## A Rapid Method to Estimate Potentially Mineralizable Nitrogen in Soil


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### ABSTRACT

**Rapid estimates of mineralizable N in soil are important for management decisions and soil quality assessments. We adapted and evaluated a rapid method based on measuring the gas pressure generated when soil is treated with $Ca(ClO)_2$ in a closed vessel. An experiment was conducted to determine the effects of reaction time, soil/reagent, and soil/water ratios on the gas pressure generated by the method. Based on this experiment, 5 g of soil, 5 mL of deionized water, 0.3 g $Ca(ClO)_2$, and a reaction time of 25 min were selected as optimum conditions. The method was evaluated with 60 Cecil (fine, kaolinitic, thermic Typic Kanhapludults) sandy loam samples ranging in organic C from 4 to 16 g C $kg^{-1}$. Nitrogen mineralized in 24 d and soil microbial biomass C (SMBC) were measured and related to the $Ca(ClO)_2$ method and to two other rapid methods, the flush of $CO_2$ during 3 d following rewetting of a dry soil, and the $NH_4$–N extractable with hot 2 *M* KCl. The $Ca(ClO)_2$ method (mmol $kg^{-1}$) was strongly correlated with net N mineralized in 24 d ($r = 0.77$) and with microbial biomass C ($r = 0.90$). The method was also correlated with the flush of $CO_2$ during 3 d following rewetting of dried soil ($r = 0.85$) and with the $NH_4$–N extractable with hot 2 *M* KCl ($r = 0.86$). These results indicate that the $Ca(ClO)_2$ method may be useful to make rapid estimates of mineralizable N and microbial biomass C in soil. Additional work is needed to investigate the nature of the compounds oxidized by the method.**


Nonlegume crop yields are determined in part by the amount of inorganic N available during the growing season. In many soils, a significant proportion of this available N is derived from mineralization of the soil organic matter (Cabrera et al., 1994). Consequently, the ability to estimate mineralizable N in soils is important to determine N fertilizer rates that optimize production without risking environmental pollution.

Although numerous biological and chemical methods have been proposed to measure potentially mineralizable N in soils (Keeney, 1982; Stanford, 1982), none of these methods is currently used by soil testing laboratories because they are too time-consuming. Modern soil testing laboratories strive for a short turnaround time and therefore need methods that require little time. Because of the time constraints of biological processes, a good rapid method (as an index of potentially mineralizable N) would likely be chemical, although recent work by Franzluebbers et al. (2000) obtained promising results with a short-term biological method. Franzluebbers et al. (2000) found that the $CO_2$-C evolved during the first 3 d of soil incubation at 25°C was highly correlated ($r^2 = 0.67$) with the amount of net N mineralized in 24 d.

A promising, chemical method is the hot 2 *M* KCl, which measures the $NH_4^+$ released from soil heated at 100°C for 4 h (Gianello and Bremner, 1986). Several studies have shown that this method can be used to estimate mineralizable N in soil (Saint-Fort et al., 1993; Jalil et al., 1996; Campbell et al., 1997). Another potentially useful, chemical method could be a method originally developed in Sweden to measure the $NH_4^+$ content of manure slurries (Chescheir et al., 1985; Piccinini and Bortone, 1991). This method is based on measuring the pressure developed when a strong oxidizing reagent such as $Ca(ClO)_2$ or NaClO oxidizes $NH_4^+$ to $N_2$ gas in a closed vessel. The pressure of the released gas, which is proportional to the $NH_4^+$ oxidized, is used to estimate the $NH_4^+$ content of manure slurries. In a study with this method, Chescheir et al. (1985) concluded that in addition to oxidizing $NH_4^+$, the method also oxidizes the readily mineralizable organic N in manure and sludge. Recently, Qafoku (1998) found that measurements made with the $Ca(ClO)_2$ method were strongly related ($r^2 = 0.70$; $p < 0.05$) to available N (mineralizable N + inorganic N) in poultry litter. These results suggest that the $Ca(ClO)_2$ method may be adaptable for estimating mineralizable N in soil. Advantages of this method over previously proposed methods would be a simple setup and a rapid analysis time (<1 h). Because of its speed, this method may also be useful for rapid soil quality assessments, in which estimates of microbial activity are usually required (Stamatiadis et al., 1999; Wander and Bollero, 1999).

The objectives of this work were: (i) to adapt the $Ca(ClO)_2$ method to soils by evaluating the effect of reaction time, soil/reagent ratio, and soil/water ratio on the gas pressure generated by the method, and (ii) to evaluate the $Ca(ClO)_2$ method in comparison with two other rapid methods (flush of $CO_2$ during 3 d of aerobic incubation following rewetting of dry soil and extractable $NH_4$-N with hot 2 *M* KCl) for their association with mineralizable N in a 24-d incubation and SMBC.


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**Abbreviations:** SMBC, soil microbial biomass C; TC, total C; TN, total N.

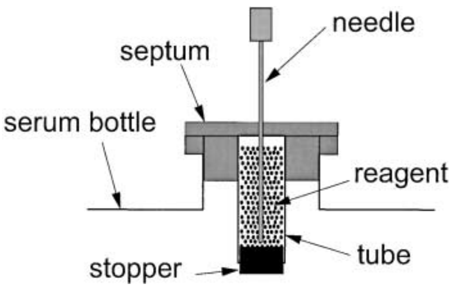


**Fig. 1. Diagram showing arrangement of rubber septum with tube containing reagent installed at the mouth of a 120-mL serum bottle.**

## MATERIALS AND METHODS

### Soils

Soil used to optimize the method was collected in August 1999 from the surface horizon of an area mapped as Cecil sandy loam in the P-1 watershed (33° 54′ N lat., 83° 24′ W long.) of the J. Phil Campbell Sr. Natural Resource Conservation Center, near Watkinsville, GA (18.8 g C $kg^{-1}$, 1.71 g N $kg^{-1}$). This 1.3-ha watershed has been under no-tillage crop production since 1974. Soil used to compare the method with other N mineralization indices was collected at a nearby tillage experiment in the spring of 1996 (Franzluebbers et al., 1999). The area is mapped as Cecil sandy loam and the samples were collected from depths of 2.5 to 7.5 and 7.5 to 15 cm. Soil was oven-dried at 55°C for 48 h, lightly crushed to pass a 4.75-mm sieve, mixed, and stored in paper bags at room temperature until analyzed. Clay content was determined by the hydrometer method and sand content by sieving (Gee and Bauder, 1986). Subsamples were ground (<153 μm) and analyzed for total C (TC) and total N (TN) by dry combustion with a LECO 2000 CNS analyzer (LECO Equipment Corp., St. Joseph, MI). It should be noted that drying and grinding the soil samples may have increased their net N mineralization during incubation (Hassink, 1992).

### Method Optimization

#### Effect of Reaction Time

Five grams of soil were mixed with 5 mL of distilled water in a 120-mL glass serum bottle (Fig. 1). A small Tygon tube (8 mm o.d., 5 mm i.d., 30 mm long, with a gray polyvinyl chloride stopper, 6 mm long, inserted in its bottom end) containing 0.3 g $Ca(ClO)_2$ was inserted inside a rubber septum that was tightly fitted to the mouth of the bottle. After placing the rubber septum on the bottle, a hypodermic needle was inserted through the septum to equalize internal and external pressures. Subsequently, the reagent was dropped into the soil suspension by pushing out the stopper at the end of the tube with a hypodermic needle inserted through the rubber septum (Fig. 1). The mixture was shaken on an orbital shaker (200 rpm) at room temperature (23°C) and pressure (relative to atmospheric pressure) was measured at 5, 10, 25, 30, 40, 50, 60, 70, 80, 90, and 100 min with a pressure transducer (model Tensimeter, Soil Measurement Systems, Tucson, AZ; range = 0.0999 MPa; sensitivity = $9.9 \times 10^{-5}$ MPa). Four replications were used in this study.

### Effect of Soil/Reagent and Soil/Water Ratios on N-Meter Pressure

A factorial combination of three soil/reagent ratios (25, 16.7, 12.5) and two soil/water ratios (1, 0.5) was used to generate six treatments, which were replicated five times. Five grams of soil were mixed with 5 or 10 mL of distilled water in a 120-mL glass serum bottle that was capped with a rubber septum as described previously. Calcium hypochlorite (0.2, 0.3, or 0.4 g) was added to the soil suspension and the mixture was shaken on an orbital shaker (200 rpm) at room temperature (23°C) for 25 min. The pressure inside the bottle was measured at 25 min with a tensimeter.

### Method Evaluation

Sixty soil samples were selected to obtain a range of organic C that varied from 4 to 16 g $kg^{-1}$ because of sampling depth and previous management (Table 1). Five grams of soil were mixed in a serum bottle (120 mL) with 5 mL of deionized water and 0.3 g of $Ca(ClO)_2$. The bottle was shaken on an orbital shaker as described previously and the pressure was measured at 25 min. Five replications were used in this study. The pressure was converted to moles of gas generated with the equation:

$$pV = nRT \quad [1]$$

where $p$ equals pressure (kPa), $V$ equals headspace volume (L), $n$ equals number of moles of gas, $R$ equals gas constant (8.3144 L kPa $mol^{-1}$ $K^{-1}$), and $T$ equals absolute temperature (298 K). At the time of pressure measurements, the temperature inside the bottles was 298 K, slightly above room temperature (296 K). Following the pressure reading, a 3-mL gas sample was taken from the headspace of each bottle to be analyzed for $CO_2$ concentration with a gas chromatograph (Varian Star 3600 CX, Varian Analytical Instruments, Sugarland, TX), which was equipped with a thermal conductivity detector (TCD) and a 3.6-m Porapak Q column (Altech Associates, Inc., Deerfield, IL). Instrument parameters included $N_2$ as carrier gas (17 mL $min^{-1}$), oven at 50°C, and detector temperature at 200°C. The moles of $CO_2$ generated by the reaction were calculated from the $CO_2$ concentration in the headspace (minus background concentration in the air) and the total number of moles of gas in the headspace. The total number of moles of gas in the headspace was calculated using Eq. [1] and the absolute pressure inside each bottle (atmospheric pressure + differential pressure measured with the tensimeter).

### Hot Potassium Chloride Extraction

Ammonium extracted by hot KCl was determined in five replicates of each of the 60 soil samples. For each replicate, 3 g of soil and 20 mL of 2 *M* KCl were placed in a 50-mL Pyrex tube, which was capped and heated at 100°C for 4 h by immersion in boiling water. At the end of 4 h, the tubes were removed from the water bath and allowed to cool until they reached room temperature. A 15-mL sample of the supernatant volume was taken and stored in a plastic vial at −13°C until analyzed for $NH_4^+$ concentration. Extractable $NH_4^+$ in cold 2 *M* KCl (3 g of soil, 20 mL of 2 *M* KCl at room temperature, reciprocating shaker at 120 rpm for 30 min, centrifuged at 180 × *g* for 20 min) was subtracted from extractable $NH_4^+$ in hot 2 *M* KCl to calculate $NH_4^+$ released by heating. Ammonium concentration was determined by the salicylate-hypochlorite method (Crooke and Simpson, 1971).

### Carbon Mineralization

Carbon mineralization was determined from the $CO_2$ evolved during a 3-d incubation in each of the 60 soil samples. Two 60-g subsamples of oven-dried soil were moistened to 50% water-filled pore space and placed into a 1-L jar, along with a vial containing 10 mL of *M* NaOH to trap the $CO_2$

**Table 1. Clay, sand, organic C, organic N, $NH_4^+$ released by hot 2 *M* KCl, net N mineralized in 24 d, $CO_2$ respired in 3 d, microbial biomass C, and gas generated by $Ca(ClO)_2$ method for 60 soil samples.**

| Sample ID | Clay | Sand | Organic C | Organic N | Hot KCl | Hot–Cold KCl | N min 24 d | $CO_2$ resp 3 d | Biomass C | Gas Generated |
|---|---|---|---|---|---|---|---|---|---|---|
| | g kg$^{-1}$ | | | | mg N kg$^{-1}$ | | | mg C kg$^{-1}$ | | mmol kg$^{-1}$ |
| 1 | 130 | 740 | 8.50 | 0.68 | 19.6 | 7.6 | 34.7 | 199 | 651 | 50.45 |
| 2 | 140 | 720 | 5.46 | 0.44 | 12.1 | 5.7 | 18.1 | 124 | 389 | 19.28 |
| 3 | 110 | 740 | 8.55 | 0.69 | 18.7 | 8.2 | 43.4 | 220 | 611 | 55.79 |
| 4 | 130 | 750 | 5.75 | 0.46 | 12.2 | 5.8 | 24.9 | 143 | 475 | 23.65 |
| 5 | 120 | 730 | 6.73 | 0.52 | 15.1 | 6.6 | 22.8 | 130 | 378 | 32.95 |
| 6 | 170 | 680 | 4.56 | 0.35 | 9.3 | 3.9 | 10.8 | 74 | 336 | 6.37 |
| 7 | 120 | 740 | 8.28 | 0.65 | 17.9 | 7.7 | 27.6 | 162 | 480 | 61.90 |
| 8 | 140 | 710 | 6.49 | 0.49 | 12.3 | 5.5 | 11.9 | 117 | 382 | 22.19 |
| 9 | 80 | 810 | 7.85 | 0.61 | 14.9 | 7.0 | 40.1 | 184 | 567 | 48.67 |
| 10 | 100 | 810 | 4.89 | 0.38 | 8.4 | 3.5 | 18.6 | 107 | 330 | 7.81 |
| 11 | 130 | 760 | 9.04 | 0.70 | 16.5 | 8.2 | 33.8 | 230 | 592 | 57.16 |
| 12 | 160 | 720 | 5.75 | 0.46 | 10.9 | 5.0 | 29.4 | 143 | 401 | 21.28 |
| 13 | 140 | 720 | 8.26 | 0.66 | 15.1 | 6.8 | 29.8 | 203 | 589 | 41.53 |
| 14 | 160 | 720 | 5.90 | 0.47 | 10.8 | 5.2 | 20.3 | 133 | 387 | 23.83 |
| 15 | 130 | 730 | 11.67 | 0.90 | 21.9 | 11.2 | 46.6 | 269 | 722 | 72.80 |
| 16 | 190 | 710 | 6.73 | 0.56 | 12.1 | 5.3 | 22.0 | 142 | 390 | 33.66 |
| 17 | 180 | 700 | 9.23 | 0.76 | 15.9 | 7.6 | 29.3 | 164 | 549 | 60.27 |
| 18 | 180 | 680 | 8.44 | 0.71 | 14.4 | 7.0 | 40.3 | 193 | 479 | 56.42 |
| 19 | 120 | 800 | 8.63 | 0.68 | 16.8 | 8.4 | 39.0 | 217 | 565 | 51.71 |
| 20 | 200 | 690 | 6.24 | 0.49 | 11.7 | 5.5 | 24.9 | 117 | 378 | 20.73 |
| 21 | 110 | 720 | 16.34 | 1.25 | 27.2 | 15.6 | 50.9 | 284 | 889 | 88.78 |
| 22 | 100 | 740 | 9.67 | 0.64 | 14.3 | 7.8 | 33.6 | 148 | 497 | 44.39 |
| 23 | 120 | 710 | 10.10 | 0.69 | 20.1 | 9.0 | 30.3 | 205 | 675 | 60.65 |
| 24 | 130 | 680 | 7.20 | 0.42 | 12.4 | 6.8 | 23.4 | 156 | 454 | 37.53 |
| 25 | 130 | 690 | 10.64 | 0.84 | 20.5 | 10.6 | 35.1 | 168 | 637 | 68.47 |
| 26 | 130 | 690 | 8.27 | 0.56 | 15.8 | 6.5 | 26.7 | 136 | 447 | 49.30 |
| 27 | 130 | 710 | 9.71 | 0.71 | 20.5 | 9.5 | 42.9 | 223 | 649 | 72.12 |
| 28 | 130 | 740 | 7.83 | 0.56 | 14.7 | 6.6 | 23.4 | 137 | 452 | 45.68 |
| 29 | 120 | 750 | 9.41 | 0.75 | 20.5 | 10.0 | 46.5 | 207 | 590 | 57.72 |
| 30 | 120 | 750 | 6.40 | 0.46 | 11.9 | 6.0 | 25.7 | 137 | 436 | 30.75 |
| 31 | 120 | 750 | 9.41 | 0.72 | 17.4 | 8.0 | 20.6 | 205 | 657 | 55.01 |
| 32 | 130 | 740 | 6.23 | 0.42 | 11.2 | 5.6 | 21.6 | 142 | 426 | 17.83 |
| 33 | 90 | 770 | 9.89 | 0.70 | 18.0 | 9.1 | 35.5 | 243 | 677 | 62.09 |
| 34 | 100 | 760 | 5.93 | 0.39 | 11.0 | 5.5 | 27.3 | 215 | 419 | 27.09 |
| 35 | 90 | 760 | 10.19 | 0.80 | 20.2 | 10.8 | 38.9 | 220 | 695 | 65.57 |
| 36 | 100 | 750 | 6.19 | 0.44 | 10.9 | 4.8 | 22.1 | 153 | 459 | 30.91 |
| 37 | 110 | 670 | 11.35 | 0.87 | 24.7 | 11.1 | 57.5 | 291 | 799 | 73.79 |
| 38 | 110 | 670 | 10.02 | 0.68 | 14.0 | 5.9 | 26.7 | 185 | 492 | 45.68 |
| 39 | 130 | 660 | 10.45 | 0.82 | 22.7 | 11.0 | 44.3 | 257 | 746 | 63.95 |
| 40 | 140 | 650 | 6.78 | 0.48 | 15.5 | 8.5 | 26.7 | 150 | 476 | 34.78 |
| 41 | 80 | 750 | 7.81 | 0.54 | 17.3 | 8.7 | 19.7 | 176 | 529 | 39.89 |
| 42 | 100 | 750 | 4.65 | 0.26 | 10.7 | 4.2 | 21.7 | 118 | 361 | 15.26 |
| 43 | 90 | 720 | 10.05 | 0.72 | 20.0 | 9.4 | 41.1 | 240 | 658 | 64.75 |
| 44 | 110 | 710 | 5.99 | 0.38 | 15.9 | 8.3 | 28.0 | 147 | 411 | 28.66 |
| 45 | 130 | 640 | 12.46 | 0.92 | 24.8 | 11.8 | 76.6 | 280 | 782 | 84.2 |
| 46 | 135 | 640 | 8.82 | 0.62 | 18.1 | 10.1 | 31.6 | 180 | 515 | 52.26 |
| 47 | 110 | 730 | 9.68 | 0.69 | 21.2 | 11.3 | 53.8 | 223 | 626 | 64.17 |
| 48 | 100 | 730 | 6.60 | 0.42 | 15.2 | 7.9 | 33.1 | 149 | 425 | 33.51 |
| 49 | 130 | 630 | 11.74 | 0.81 | 24.1 | 13.7 | 58.1 | 227 | 674 | 75.60 |
| 50 | 130 | 630 | 8.81 | 0.55 | 16.6 | 7.7 | 41.7 | 166 | 486 | 48.87 |
| 51 | 90 | 710 | 9.87 | 0.65 | 20.0 | 10.3 | 27.4 | 214 | 677 | 58.25 |
| 52 | 110 | 700 | 6.97 | 0.38 | 13.2 | 5.6 | 20.7 | 130 | 443 | 29.79 |
| 53 | 140 | 610 | 13.85 | 1.02 | 23.5 | 11.8 | 37.0 | 299 | 797 | 84.21 |
| 54 | 150 | 600 | 10.21 | 0.66 | 16.7 | 8.3 | 40.0 | 184 | 535 | 64.87 |
| 55 | 130 | 690 | 10.07 | 0.71 | 20.8 | 9.7 | 35.3 | 207 | 640 | 72.46 |
| 56 | 160 | 650 | 6.61 | 0.41 | 14.7 | 6.8 | 20.4 | 142 | 425 | 28.41 |
| 57 | 170 | 550 | 12.90 | 0.95 | 23.6 | 11.5 | 48.0 | 265 | 777 | 87.27 |
| 58 | 170 | 440 | 10.43 | 0.71 | 20.2 | 10.2 | 38.4 | 250 | 554 | 69.78 |
| 59 | 160 | 520 | 14.19 | 0.97 | 24.2 | 8.0 | 32.1 | 193 | 741 | 90.76 |
| 60 | 170 | 500 | 13.28 | 0.90 | 22.3 | 11.3 | 52.0 | 254 | 611 | 78.22 |

evolved, and a vial with water to maintain humidity. Jars were sealed and incubated in the dark at 25°C for 3 d. Following incubation, the vial of alkali was removed and the amount of $CO_2$ determined by back titration with 1 *M* HCl following addition of excess $BaCl_2$ (Anderson, 1982).

## Nitrogen Mineralization

Potential N mineralization was determined by measuring the inorganic N ($NO_3^-$–N + $NO_2^-$–N + $NH_4^+$–N) produced during a 24-d incubation at 25°C and 50% water-filled pore space, as previously reported in Franzluebbers (1999). Inorganic N was extracted by shaking a 10-g subsample with 20 mL of 2 *M* KCl for 30 min. The concentrations of $NO_2^-$ and $NO_3^-$ in the extract were determined after reduction of $NO_3^-$ to $NO_2^-$ with a Cd column (Keeney and Nelson, 1982). The concentration of $NH_4^+$ was determined by the salicylate-hypochlorite method (Crooke and Simpson, 1971).

## Microbial Biomass

A 60-g dried subsample was rewetted to 50% water-filled pore space and preincubated at 25°C for 10 d. At 10 d, soil was fumigated with chloroform for 24 h, vapors removed,

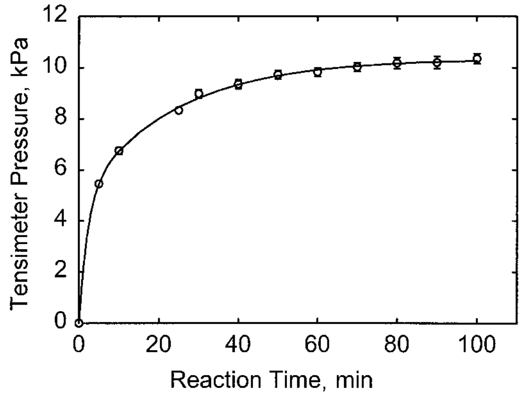


**Fig. 2. Effect of reaction time on pressure developed by the $Ca(ClO)_2$ method (mean and standard deviation).**

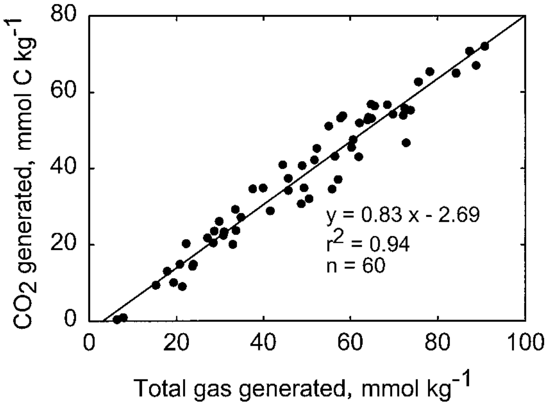


**Fig. 4. Relationship between total gas and total $CO_2$ generated by the $Ca(ClO)_2$ method.**

sample placed into a 1-L jar along with vials of alkali and water as described previously, and incubated at 25°C for 10 d (Jenkinson and Powlson, 1976). Soil microbial biomass C was calculated as the quantity of $CO_2$–C evolved following fumigation divided by an efficiency factor of 0.41 as reported previously by Franzluebbers et al. (1999).

### Statistical Analyses

A two-way analysis of variance was performed with the data collected in the experiment on soil/reagent and soil/water ratios (SAS Institute, 1994). Least significant differences ($p <$ 0.05) were calculated to determine differences among treatments. Correlation coefficients were calculated to evaluate the association between the different variables measured.

## RESULTS AND DISCUSSION

### Method Optimization

#### Effect of Reaction Time

Increasing the reaction time from 5 to 100 min increased the pressure from 5.4 to 10.3 kPa (Fig. 2). The pressure measured at 25 min was 8.3 kPa, which corresponded to 80% of the pressure measured at 100 min. Because we were interested in developing a routine soil test to be performed as rapidly as possible, we selected 25 min as the reaction time for further measurements.

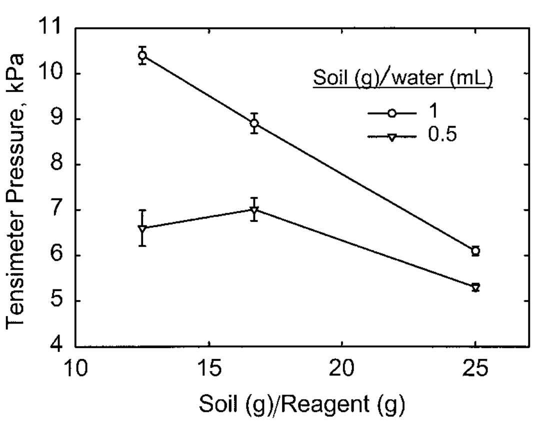


**Fig. 3. Effect of soil/reagent (g/g) and soil/water (g/mL) ratios on the pressure developed by the $Ca(ClO)_2$ method (mean and standard deviation).**

#### Effect of Soil/Reagent and Soil/Water Ratios

Decreasing the soil/reagent ratio from 25 to 16.7 increased the pressure by a larger value at a soil/water ratio of 1 than at a soil/water ratio of 0.5 (Fig. 3). Similarly, when the soil/reagent ratio was decreased from 16.7 to 12.5, the pressure increased at a soil/water ratio of 1 but did not change ($p < 0.001$) at a soil/water ratio of 0.5. Thus, at a given soil/reagent ratio, pressure response was greater with less water, which was probably a function of low gas diffusion through water. Based on these results, we selected a soil/water ratio of 1 for the method. In spite of obtaining larger pressures at a soil/reagent ratio of 12.5 than at 16.7, we selected the latter because it was difficult to push the needle through 0.4 g of reagent (12.5 ratio) to remove the bottom stopper and allow the reagent to mix with the soil/water suspension. It should be kept in mind, however, that soils with high organic C contents may require a smaller soil/reagent ratio to ensure complete oxidation of labile C and N compounds.

### Method Evaluation

Total C and N in the 60 soil samples varied 30% around the mean, that is 8.7 ± 2.5 and 0.63 ± 0.19 g $kg^{-1}$, respectively. Variation in SMBC was lowest at 24% and variation in the $Ca(ClO)_2$ method was greatest at 44%.

Because $NH_4^+$ is converted to $N_2$ gas by the $Ca(ClO)_2$ method, a relatively high initial $NH_4^+$ content in the soil could have a significant effect on the results obtained. By calculation, 1 mmol of $NH_4^+$–N $kg^{-1}$ (14 mg N $kg^{-1}$) is converted to 0.5 mmol $N_2$ $kg^{-1}$. In our samples, the average initial $NH_4^+$ content was small (8 mg N $kg^{-1}$) and as a result the contribution of the initial $NH_4^+$ to the millimoles of gas generated was <1%. Although this is likely to be the case with most soils because nitrification quickly converts $NH_4^+$ to $NO_3^-$, the initial $NH_4^+$ concentrations should be taken into consideration when using this method.

Approximately 83% of the moles of gas generated during the 25-min reaction was made up of $CO_2$ (Fig. 4). Because the remaining 15% is likely to be made up mostly of $N_2$ gas (of which only 1% was derived from the initial $NH_4^+$ in the soil), these results suggest that

**Table 2. Correlation between the different indices measured ($n = 60$; $p < 0.0001$ for all correlation coefficients).**

| Index | $CO_2$ 3 d | Hot KCl | Cold KCl | Hot-Cold KCl | N min 24 d | SMBC† | TC‡ | TN§ |
|---|---|---|---|---|---|---|---|---|
| $Ca(ClO)_2$ | 0.85 | 0.93 | 0.88 | 0.86 | 0.77 | 0.90 | 0.95 | 0.93 |
| $CO_2$ 3 d | | 0.85 | 0.75 | 0.83 | 0.80 | 0.90 | 0.83 | 0.83 |
| Hot KCl | | | 0.93 | 0.94 | 0.79 | 0.92 | 0.91 | 0.89 |
| Cold KCl | | | | 0.74 | 0.68 | 0.86 | 0.84 | 0.82 |
| Hot-Cold KCl | | | | | 0.79 | 0.86 | 0.86 | 0.84 |
| N min 24 d | | | | | | 0.74 | 0.73 | 0.72 |
| SMBC | | | | | | | 0.90 | 0.91 |
| TC | | | | | | | | 0.96 |

† SMBC = soil microbial biomass C.
‡ TC = total C.
§ TN = total N.

the organic compounds oxidized by the $Ca(ClO)_2$ method have a relatively low C/N ratio.

The amount of gas generated by the method was strongly correlated ($p < 0.0001$) with net N mineralized in 24 d ($r = 0.77$; Table 2) and with SMBC ($r = 0.90$). The flush of $CO_2$ during 3 d following rewetting of dry soil was also strongly related to net N mineralized in 24 d ($r = 0.80$) and to SMBC ($r = 0.90$). Similar relationships for the short-term flush of $CO_2$ have been documented previously in other soils from Georgia (Franzluebbers et al., 1999), as well as in soils from Texas, Alberta, British Columbia, and Maine (Franzluebbers et al., 2000). Ammonium extractable by hot 2 *M* KCl was strongly correlated with net N mineralized in 24 d ($r = 0.79$) and with SMBC ($r = 0.86$). Gianello and Bremner (1986) also found a strong relationship between $NH_4^+$ released by hot 2 *M* KCl and potential N mineralization in 30 Iowa soils ($r = 0.96$). Because the $Ca(ClO)_2$ method was closely associated with net N mineralized in 24 d and SMBC, as well as with the short-term flush of $CO_2$ ($r = 0.85$) and $NH_4^+$ extractable by hot 2 *M* KCl ($r = 0.86$), these results suggest that the $Ca(ClO)_2$ method may be useful to make rapid estimates of mineralizable N and microbial biomass in soil.

## CONCLUSIONS

We were able to optimize conditions for the development of the $Ca(ClO)_2$ method as a soil testing tool. Reaction time of 25 min released 80% of the gas produced in 100 min. Equal portions of soil (g) and water (mL) produced better responses than a lower ratio of soil/water. A soil/reagent ratio of 16.7 was more responsive than a higher ratio and was at the lower limit for practical considerations for getting reagent mixed with soil. The $Ca(ClO)_2$ method response was highly correlated with net N mineralized in 24 d and SMBC, as well as with other proposed indices of biological soil quality, including the flush of $CO_2$ during 3 d of aerobic incubation following rewetting of dried soil and the $NH_4^+$ extractable by hot 2 *M* KCl. The $Ca(ClO)_2$ protocol is rapid and could be a useful tool to make rapid estimates of mineralizable N and SMBC. Comparison of the $Ca(ClO)_2$ method with potential N mineralization in other soils and under varying management should be investigated because results from this study indicate great potential for using the method as a rapid soil quality assessment tool. Future work should also investigate the relationship between the $Ca(ClO)_2$ method response and N uptake by plants in the field. Additional work is needed to further investigate the nature of the compounds oxidized by the method.

## REFERENCES


Anderson, J.P.E. 1982. Soil respiration. p. 83–871. *In* A.L. Page at al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. SSSA, Madison, WI.

Cabrera, M.L., M.F. Vigil, and D.E. Kissel. 1994. Potential nitrogen mineralization: Laboratory and field evaluation. p. 15–30. *In* J. Havlin et al. (ed.) Soil testing: Prospects for improving nutrient recommendations. SSSA Spec. Publ. 40. SSSA, Madison, WI.

Campbell, C.A., Y.W. Jame, A. Jalil, and J. Shoenau. 1997. Use of hot KCl-$NH_4$-N to estimate fertilizer N requirements. Can J. Soil Sci. 77:161–166.

Chescheir, G.M. III, P.W. Westerman, and L.M. Safley, Jr. 1985. Rapid methods for determining nutrients in livestock manures. Trans. ASAE 28:1817–1824.

Crooke W.M., and W.E. Simpson. 1971. Determination of ammonium Kjeldahl digest of crops by an automated procedure. J. Sci. Food Agric. 22:9–10.

Franzluebbers, A.J. 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol. Biochem. 31:1083–1090.

Franzluebbers, A.J., R.L. Haney, C.W. Honeycutt, H.H. Schomberg, and F.M. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613–623.

Franzluebbers, A.J., G.W. Langdale, and H.H. Schomberg. 1999. Soil carbon, nitrogen, and aggregation in response to type and frequency of tillage. Soil Sci. Soc. Am. J. 63:349–355.

Gee, G.W., and J.W. Bauder. 1986. Particle size analysis. p. 383–411. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9 SSSA, Madison,WI.

Gianello, C., and J.M. Bremner. 1986. Comparison of chemical methods of assessing potentially mineralizable organic nitrogen in soil. Commun. Soil Sci. Plant Anal. 17:215–236.

Hassink, J. 1992. Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils. Biol. Fertil. Soils 14: 126–134.

Jalil, A., C.A. Campbell, J. Schoenau, J.L. Henry, Y.W. Jame, and G.P. Lafond. 1996. Assessment of two chemical extraction methods as indices of available nitrogen. Soil Sci. Soc. Am J. 60:1954–1960.

Jenkinson, D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. I. Fumigation with chloroform. Soil Biol. Biochem. 8:167–177.

Keeney, D.R. 1982. Nitrogen—Availability indices. p. 711–733. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen–Inorganic forms. p. 643–698. *In* A.L. Page et al. (ed.) Methods of soils analysis. Part 2. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Piccinini, S., and G. Bortone. 1991. The fertilizer value of agricultural manure: Simple rapid methods of assessment. J. Agric. Eng. Res. 49:197–208.

Qafoku, O.S. 1998. Rapid methods to determine mineralizable nitrogen in poultry litter. M.S. Thesis, Crop and Soil Sciences, University of Georgia, Athens, GA.

Saint-Fort, R., J. Sheppers, and C.S. Holzhey. 1993. Comparison of potentially mineralizable nitrogen using electro-ultrafiltration and some other procedures. J. Environ. Sci. Health A28(6):1171–1183.

SAS Institute. 1994. SAS/STAT user's guide, Version 6, 4th ed. Cary, NC.

Stamatiadis, S., J.W. Doran, and T. Kettler. 1999. Field and laboratory evaluation of soil quality changes resulting from injection of liquid sewage sludge. Appl. Soil Ecol. 12:263–272.

Stanford, G. 1982. Assessment of soil nitrogen availability. p. 651–688. *In* F.J. Stevenson et al. (ed.) Nitrogen in agricultural soils. Agron. Monogr. 22. ASA and SSSA, Madison, WI.

Wander, M.M., and G.A. Bollero. 1999. Soil quality assessment of tillage impacts in Illinois. Soil Sci. Soc. Am. J. 63:961–971.